

EXPERIMENTAL NICKEL CONTACT SENSITIZATION IN MAN*

JAMES J. VANDENBERG, M.D. AND WILLIAM L. EPSTEIN, M.D.

Nickel dermatitis constitutes a difficult clinical problem. Its etiology and pathogenesis remain uncertain. Current concepts view the disease as an example of contact-type delayed hypersensitivity, but an immunologic basis has not been firmly established. In fact, Rostenberg *et al* (1) have argued persuasively against this idea; they suggest an inborn enzymatic defect to explain the disease.

In the main, previous experimental work has not taken into account the irritating qualities of nickel solutions. Many studies are negated by the fact that frankly irritating evocative patch testing preparations were used. In addition, no one has pre-tested their subjects to detect and eliminate previously sensitized persons.

MATERIALS

The subjects were 178 healthy male volunteers†. The sensitizing agent was 25 per cent nickel chloride (NiCl_2) dissolved in water with 0.1 per cent sodium lauryl sulfate (SLS) to aid penetration. The eliciting solutions were 1, 2, 5, and 10 per cent NiCl_2 and nickel sulfate (NiSO_4) in water.

Experiment I—Irritation

A survey of the literature was confusing as to the proper concentration of nickel salts for patch testing. To assess previous work we first studied the irritant properties of NiCl_2 . Nickel chloride in concentrations of 1, 2, 5, and 10 per cent aqueous solutions was dropped onto 1.5 cm gauze squares and the saturated patches were applied to the backs of 39 subjects. The test sites were completely occluded for 24, 48, and 96 hours and examined 10 to 15 minutes and 5 days after removal of the patches.

The results (Fig. 1) indicate that irritation is

directly proportional to concentration and duration of application. Clearly, 10 per cent NiCl_2 with occlusion causes too much irritation to have value as a predictive patch test. Fisher and Shapiro (2) drew similar conclusions from their studies. The 5 per cent occluded patch test also showed significant irritation; it cannot be used effectively either.

On the other hand, non-occluded patches covered only with a band-aid (Fig. 2) in the same subjects showed almost no reactions after 48 hours:

| Irritant Patch Test Reactions Without Occlusion | | | | |
|--|------|------|------|-----------|
| | 1% | 2% | 5% | 10% |
| 48 hours | 0/39 | 0/39 | 0/39 | 3/39 (8%) |

These data suggest that such a test with 5 per cent NiCl_2 solution ordinarily will not be a primary irritant. However, we found that 0.1 per cent SLS added to the 5 per cent NiCl_2 solution resulted in increased irritation:

Positive Reactions with Occlusion

| With 0.1 per cent SLS | Without 0.1 per cent SLS |
|-----------------------|--------------------------|
| 20/38 = 53 per cent | 11/38 = 29 per cent |

We adopted the 5 per cent NiCl_2 patch covered only with a band-aid (non-occluded) as the standard for our sensitizing experiments.

Experiment II—Sensitization

We next undertook a deliberate attempt to sensitize previously non-reactive normal human volunteers. All subjects were pre-tested at least once with non-occluded patches of 5 per cent NiCl_2 solution. Six positive reactors were detected and removed from the study. Clinical experience in the United States led us to believe that nickel is a weak sensitizer, so we utilized the "triple freeze" technic for sensitization (3). This method utilizes several factors which enhance sensitization, i.e. irritation, occlusion, and repeated exposure on the same limb to bombard the same regional nodes with a maximum amount of allergen. Briefly, a 3 cm circle of skin on the upper arm was frozen for 3 seconds with dichlorodifluoromethane (Freon-12). A Lintine disc* saturated with 25 per cent NiCl_2 and 0.1 per cent SLS solution was applied

* From the Division of Dermatology, Department of Medicine, University of California School of Medicine, San Francisco Medical Center, San Francisco, California.

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† Dr. Herman Gross of Neumiller Hospital, San Quentin Prison, and Dr. William Keating of Vacaville Medical Facility cooperated in the study. Glenn Watson and E. N. Murray assisted in the study.

* Johnson and Johnson.

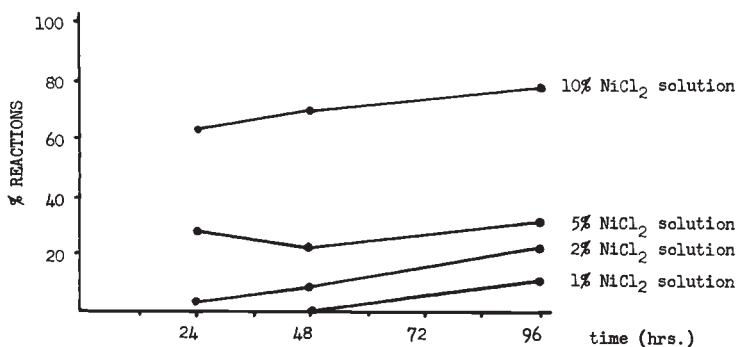


FIG. 1. Per cent of irritancy reactions related to concentration and duration of exposure. Clearly 5 per cent NiCl_2 with occlusion causes too much irritation to be used as a standard for patch testing.

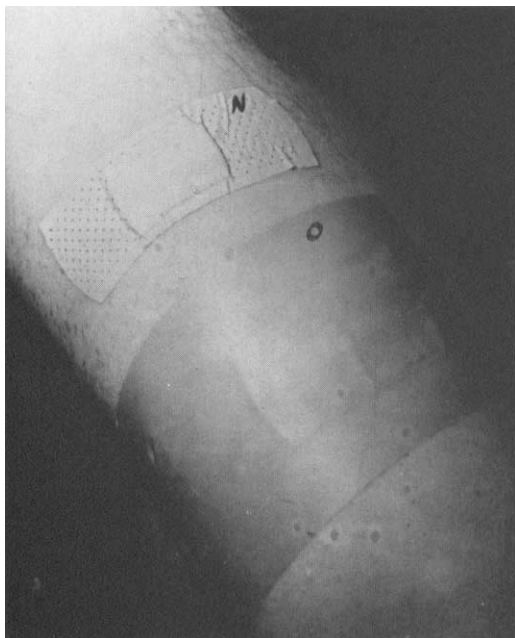


FIG. 2. Examples of the occluded (O) and non-occluded (N) patches used in these studies.

immediately to the frozen area and then occluded firmly with plastic tape for 2 days. This procedure was performed three times at 5 day intervals. Standard eliciting patch tests were applied to the opposite forearm 10 days after the third freeze and observed at 2, 3, and 5 days.

The results showed:

Per cent Sensitized

$$16/172 = 9\%$$

A number of other subjects gave questionable reactions, but repeated tests with 1, 2, and 5 per cent NiCl_2 indicated that only these 16 subjects

had been truly sensitized. These subjects were then compared by serial patch testing with the 6 sensitive subjects who had been removed from the study. No significant differences in depth or degree of reactivity were observed.

Clinically the latent period in nickel sensitivity appears to be quite long with prolonged and repeated metal contact required before the onset of the dermatitis. We wondered if a second "triple freeze" would result in a greater frequency of sensitization. Twenty negative reactors previously exposed to nickel by the "triple freeze" technic were re-exposed 4 months later by the same method. One subject was found to have developed sensitivity in the interim. The results of the second "triple freeze" were:

Percent Sensitized

$$5/19 = 26\%$$

Clearly, prolonged exposure raises the frequency of sensitization to weak allergens such as nickel.

Experiment III—Cross Sensitivity

The status of cross-sensitivity between other metals and nickel is not settled. We assessed reactivity to 2 per cent and 1 per cent cobalt chloride (CoCl_2), 1 per cent and 0.5 per cent copper sulfate (CuSO_4), and 0.5 per cent and 0.1 per cent potassium dichromate ($\text{K}_2\text{Cr}_2\text{O}_7$) in eight subjects who had developed nickel sensitivity. The test sites were examined at 2 and 5 days. One subject showed a primary irritant response to each concentration of CuSO_4 , but no other reactions occurred.

Clinical Status of Subjects

It should be emphasized that none of our subjects, either those previously sensitized or those

experimentally sensitized, demonstrated nickel dermatitis to his watch, watchband, or identification tags. The subjects apparently have remained subclinically sensitive during 6 months of observation.

DISCUSSION

From these data we conclude that sensitization to nickel can be accomplished under carefully controlled conditions. The controversial findings reported in the literature probably reflect lack of adequate controls. Despite enthusiastic reports of sensitization in guinea pigs (4-6), these have not been confirmed (7, 8) and most workers, including us, believe the reactions seen were irritant rather than allergic in nature (9).

Haxthausen (10) and Burkhardt (11) each reported no difficulty in sensitizing a few subjects to 25 per cent NiSO_4 . These results have not been generally accepted for a number of reasons. They used 10 per cent NiSO_4 for evocation and as we and others (9, 12) have shown this causes primary irritation. Also, they failed to test their subjects for prior sensitivity, and repeated tests were not used to rule out spurious responses. However, it is clear from their data that certain subjects were truly sensitive to nickel; their experimental design, unfortunately, does not reveal how this was accomplished.

It is not surprising, therefore, that a thoughtful analysis of the problem led Rostenberg *et al* (1) to conclude that nickel sensitivity is not an allergic response. They believe it represents an "enzymatic interference in a biochemically deviant person." The argument rests on analogy and negative correlations. We present positive data supporting the concept of delayed hypersensitivity as the cause of nickel dermatitis. We have ruled out the possibility of primary irritation as a factor; and repeated pre-testing eliminated previously sensitive persons. Furthermore, the frequency of reactivity was increased by repeated sensitizing exposures; this would not be expected in a biochemically deviant population. Finally, patch tests with weak dilutions of nickel salts months after sensitization still gave positive results. Hence, only positive transfer experiments remain to be done to make certain that nickel dermatitis is a variety of delayed hypersensitivity.

One unexplained aspect of nickel dermatitis is its high incidence noted in Europe (13-15) and

the relatively low frequency in the United States (2). Our findings suggest that a substantial number of Americans have the ability to develop nickel sensitivity. Indeed, six subjects removed from the study had a previously acquired sensitivity. Yet no one showed a clinical eruption.

Marcussen (16) believes subclinical reactors form a small percentage of the total. Calnan states that patch test reactivity often is not correlated with clinical disease (17). We also suggest the percentage is much higher. The situation in poison ivy-oak sensitivity offers a pertinent analogy. Here, about 50 per cent of the population is clinically sensitive, but another 35-40 per cent are subclinically sensitive and may become reactive at any time (18). Perhaps Rostenberg *et al* (1) are correct; some factor other than hypersensitivity is required to precipitate the curious pattern of clinical disease (12). Whether genetics or some other factor play this role remains to be determined.

The final point of discussion concerns cross-sensitivity to other metals such as cobalt, copper, and chromium. Stephan Epstein and others (19-23) believe significant cross-sensitivity exists, but the view is not generally held (14). Marcussen (14) points out that correction for occupational double exposure gives no support to the concept of cross-sensitivity. In our studies experimental sensitization to nickel engendered *no* reactivity to other metals. We agree with Marcussen and others that cases of dual metal hypersensitivity undoubtedly arise from multiple primary sensitizations.

SUMMARY

1. Nickel chloride solutions are very irritating when applied to human skin with occlusion. A 5 per cent solution causes no irritation when applied without occlusion under a band-aid for 48 hours. This preparation can be used for evocative patch testing. However, we emphasize that a 5 per cent solution *with* occlusion is irritating.

2. Contact-type delayed hypersensitivity can be induced experimentally in man. No cross-sensitivity to other metals was demonstrated under these conditions.

3. Discussion includes consideration of the intriguing fact that no experimentally sensitized subject demonstrated clinical sensitivity to

the metal in his ordinary environment, such as watch, watchband, or identification tags.

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DISCUSSION

DR. RICHARD L. DOBSON (Chapel Hill, N. C.): This is an important contribution to a common clinical problem.

The incidence of sensitization was reported to be 9%. Since nickel dermatitis is a problem in the atopic individual almost exclusively and since the incidence of atopy in the general population is thought to be about 10%, I wonder if the authors were able to correlate an atopic history with the ability to sensitize?

Secondly, the problem was raised about the sensitized individuals not being clinically reactive. I assume that all their subjects were males. Since women have nickel dermatitis more commonly than men, the lack of clinical disease in their subjects may not reflect a lack of sufficient sensitivity but rather reflects perhaps, the degree of exposure to the metal in clothing, for example.

DR. ADOLPH ROSTENBERG, JR. (Chicago, Illinois): Doctors Vandenberg and Epstein have apparently produced an alteration in cutaneous

reactivity to nickel. The question is, what is the proper label for this alteration? This is not just a point in semantics, in that labels betoken mechanism. They interpreted the alteration as the development of an allergic sensitization, and this may be correct; but I think that the matter has not been unequivocally settled.

Elsewhere* my colleagues and I have questioned the propriety of the label "allergic sensitization" for eczematous reactions to certain metals, in that these do not conform to the classic delayed types of sensitivity developed by simple chemicals, such as picryl chloride, 2:4 dinitrochlorobenzene, etc. The eczematous reactions produced by certain metals differ in that (1) it is not possible to correlate structure

* Rostenberg, A.; Perkins, A. J.; and Fox, J. M.: Eczematous Reactions to Nickel, Cobalt, and Chromium in Dermatoses due to Environmental and Physical Factors. Ed. by Rees R. B. Rees. Thomas. Springfield, 1962, P. 250.

and reactivity; (2) the reactions are often non-specific, i.e., a person will react to more than one metal; (3) it is not possible to transfer the reactivity; (4) the reactive and the normal person fail to show a difference with respect to the absorption of the metal; and (5) it has not been possible to "desensitize". In general, classical delayed eczematous sensitizations to simple chemicals exhibit all of points (1) to (5). The absence of any one of these points is not crucial, but the sum total argues strongly against the allergic interpretation for the pathogenesis of the metal eczemas.

DR. M. B. SULZBERGER (Washington, D. C.): I would like to comment not only on this paper but also on Dr. Rostenberg's discussion of the paper, if I may.

First of all, I would like to say that I have never quite understood Dr. Rostenberg's explanation of immunologic phenomena with incubation periods, accelerated responses, etc., as simply belonging to genetically deviant individuals. Of course, the people who get sensitized more easily are deviant in a certain way from the ones who do not get sensitized easily. Immunologic reactions also usually depend upon genetically determined degrees of susceptibility to immunologic alterations so there is no quarrel with the idea that certain forms of genetic deviation are actually the basis of an immunologic acquired alteration.

The other point I would like to comment on is that it has not been my clinical impression that the contact type of allergic eczematous dermatitis due to nickel is substantially more common in the atopic individuals than it is in the nonatopic group. It is true that one gets positive patch-test reactions with 5 per cent solutions of nickel sulfate in atopic dermatitis, i.e., in individuals with an atopic skin, much more commonly than one does if one tests normals, so-called normal skins. I believe Steiner was the first one to demonstrate this, and after that, studies were done at our school by Joseph Goodman and then by Charles Miller, Arthur Hyman, and others. All these studies showed the reaction to be not an allergic response to nickel but a primary-irritant response. It is apparent that the threshold of the atopic skin for responses of primary irritation to quite a series of substances, including nickel salts, arsenicals, iodides, feather and house dust allergens, and others, which do not produce reactions in so-called normal skins, is lower in the

atopic skin. The clinical appearance and the histologic appearance of these reactions to nickel are quite different from the real contact type of allergic eczematous responses which are on an immunologic basis. These primary irritant responses generally originate around the mouths of the follicles and are much more prone to predominantly polymorphonuclear leukocytic infiltrations and to clinically papulo-pustular lesions than are the allergic contact reactions.

One of the prime difficulties in coming to a decision as to the proper label for a reaction is that there are no absolute criteria for asserting that the reaction is on an allergic basis. In allergic reactions of the immediate type serologic evidence may furnish proof. In the delayed type the best evidence is the cellular transfer, which, in man, has not been uniformly accomplishable.

What other interpretation can be given to the altered reactivity seen? It will be recalled that, in order to produce the alteration, the authors first had to develop a fair degree of irritation at the experimental site. It has been shown that the development of a primary irritation has an effect on the skin at other sites. This is not only to be a matter of clinical observation but has been experimentally verified. Doctors Haeberlin and Fox (Primary Irritation of the Skin. A.M.A. Archives of Dermatology **80**: 690-699, December, 1959), in our laboratory, have shown this in the guinea pig. In order to check on this hypothesis, it would be worthwhile for the authors to determine whether the subjects who displayed the altered reactivity to nickel also displayed an altered reactivity to borderline primary irritant concentrations of other materials. A final test as to the nature of the reaction developed would be to see if it could be experimentally transferred to normal recipients with cells from the subjects in whom the altered reactivity was developed.

As to the question raised concerning the distinction between a reaction brought about by a genetically induced biochemical deviation and an allergic sensitization, I believe these are quite separate phenomena and that it is important to keep them separate. In a genetically induced biochemical deviation, one has an individual in whom there is a deficiency of an enzyme system brought about because of some difference in the genetic makeup of that person. This deficiency can be of any degree. The consequences of this deficiency may only be manifest when an appro-

priate stress stimulus is met, *e.g.*, persons who have a deficiency of glucose-6-phosphate dehydrogenase behave perfectly normally until they meet a drug such as primaquin. So far as is known, there is no way to transfer this genetic deficiency to other persons, nor is there any way to induce it in a person who does not possess it. On the other hand, genetic factors play a role in the development of an allergic sensitization, in that if a person is not of the appropriate genetic stock they probably cannot become sensitized to a given substance; but with many "strong sensitizers", *e.g.*, poison ivy, 2:4 dinitrochlorobenzene, foreign sera, apparently genetic factors play a minimal role, in that almost any person can become sensitized. Once a person has become sensitized, there is either serological or cellular evidence for that sensitization, and it is passively transferable to normal persons, either by means of serum or by means of living cells. Furthermore, once a sensitization has developed, the mechanism is the same for a given type of sensitization, regardless of the substance to which the person is sensitized; whereas, in a genetically induced biochemical deviation the mechanism in each instance is contingent on the specific enzyme involved.

DR. JAMES J. VANDENBERG (in closing): I wish

to thank the discussers for their cogent remarks. In answer to Dr. Dobson, our volunteers were prisoners; they have almost no history of atopy. I agree with Dr. Sulzberger that the response in atopics probably represents primary irritancy.

I cannot comment about responses in women. We studied only men.

Dr. Rostenberg's comments were very much appreciated. As I said, we induced nickel hypersensitivity but did not produce clinical disease. These men still tolerate their watch bands and identification bracelets. We wonder if the development of clinical disease may depend upon some biochemical deviant. In fact, this may be the difference between the person who becomes sensitized and the one who becomes sensitized and then develops clinical dermatitis. The status of subclinical reactors has not been thoroughly explored in the case of nickel sensitivity.

In regard to the possibility of false-positive reactions because of extreme inflammation of the skin elsewhere, I should point out that the hypersensitivity persisted long after clinical healing occurred. Patch tests 6 months later still gave strongly positive reactions. We have induced an altered tissue reactivity that presumably is immunologic in nature. Passive transfer experiments are under way at the present time, and I hope to report on them later.